

EARLY STAGE TREATMENT OF COMPARTMENT SYNDROME USING POLYMER - SOL-GEL COMPOSITE GROWTH FACTOR DELIVERY WOUND DRESSINGS

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ABSTRACT

Compartment syndrome (CS) as a result of blast or traumatic injury is a devastating problem in the battlefield. The ultimate goal of this study is to develop an integrated toolkit of novel, biodegradable wound dressing composites for early stage treatment of CS. Composites made from the tyrosine-based block copolymers and silica based sol-gels were designed as an absorbent to remove fluid from injured muscle compartments and as wound dressings to deliver therapeutic agents to the compartment.

This *in vitro* study reports the controlled release of protein analog and antibiotics from the room temperature processed sol-gel particles. Furthermore, this study reports the fabrication of the composite wound dressings with mechanical properties for the clinical application.

1. INTRODUCTION

CS occurs when elevated intramuscular pressure decreases vascular perfusion of a muscle compartment to a point no longer sufficient to maintain viability of the muscle and neural tissue contained within the compartment. The etiology of CS as it results from the high energy blast injuries sustained by our war fighters in the current conflict in the Middle East is not well established, yet deserves thoughtful preventative action. If left untreated or diagnosed late, CS leads to amputation or death.

Extensive studies showed that even though significant progress has been made in the understanding of CS, there is still no effective way for early treatment. It becomes apparent that a new approach is needed. Given the current state and recognizing the clinical needs, we propose to develop early stage treatments specifically addressing the etiology associated with high energy explosive device (IED) injuries that produce the clinical signs of compartment syndrome. It is our hypothesis that treatments can act prophylactically and reduce the need for fasciotomy.

For this purpose, our ultimate goal is to create a wound dressing composed of controlled release sol-gel powders with unique release kinetics in a tyrosine-based block copolymer film. The goal is to achieve two critical functions in early stage treatments: 1) deliver pro-angiogenic factors and antibiotics that counteract the injurious events associated with high energy explosive device (IED) injuries; 2) absorb extracellular fluid within the compartment to reduce the hydrostatic pressure increase. The delivery of therapeutic agents will be achieved by incorporating molecules into sol-gel particles that are embedded in the copolymer film. Herein we report on our initial studies quantifying the release of model molecules simulating large pro-angiogenic factors. We selected as molecular analogs dextran molecules. Thus, we report on the controlled release of dextran of the same molecular size as pro-angiogenic factors and report on actual antibiotics release kinetics as well as the fabrication of the composite wound dressings with mechanical properties as required for the clinical application.

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2. MATERIALS AND METHODS

2.1. Sol-gel formulations for the controlled delivery of growth factor analog

Dextran-Texas Red conjugated molecules, with molecular weight (MW) of 10,000, 40,000 and 70,000 (Molecular Probes) were incorporated in silica sol-gels which were synthesized using either an acid catalyzed hydrolysis or a two-step acid-base catalyzed hydrolysis of tetraethoxysilane (TEOS, Strem Chemicals, Newburyport, MA). The synthesis process was designed keeping in mind the sensitivity of dextran to pH: both acid catalyzed and acid - base catalyzed sols were maintained at a pH equal to or above 3 prior to incorporation of the dextran molecules. 10 mg/g of dextran of various MW (dextran/SiO₂, %, w/w) were added to the liquid sols having a water/TEOS ratio (R) of 8. After casting, condensation and drying, sol-gels were ground and sieved to produce granules in a size range of 150 to 350 μ m. The handling and storage procedures of dextran and sol-gels with dextran were designed keeping in mind the sensitivity of these molecules to light and temperature. *In vitro* release was studied in phosphate buffered saline (PBS, pH=7.4) at 37°C. The release of dextran was measured using a UV/visible light spectrophotometer at 595 nm.

2.2 Sol-gel formulations for the controlled release of antibiotics

Sol-gel derived silica microspheres were synthesized using an acid-base catalyzed hydrolysis of TEOS followed by emulsification. TEOS, de-ionized (DI) water and 0.1 M HCl were mixed to form an acid-catalyzed sol with R of 5, 6 or 8. Sols with 20 mg/g Vancomycin (Abbott Labs, Chicago, IL) were prepared by adding corresponding amounts of the drugs. At R smaller than 5, immediate precipitation of the drugs was observed. This suggested that, in contrast to previously used water-free sol-gel synthesis of microspheres (Peterson et al., 1998), incorporation of drugs requires the presence of water at R equal to or greater than 5. The base, 0.08 M NH₄OH, was added dropwise to the sol, and then, prior to gelation the sol was applied to stirring vegetable oil. Microspheres formed only when the time to gelation of acid-base catalyzed sol was 20-40 minutes. Precipitated microspheres were separated from the oil and then rinsed with DI water and alcohol. The acid-base catalyzed sols were also used to produce ground granules (210-500 μ m).

Morphology and the size distribution of the microspheres were determined microscopically using an image analysis system (Image-Pro Plus 4.0).

In vitro release was studied in PBS at 37°C with daily solution exchange. The release of vancomycin was

measured spectrophotometrically at 280 and 265 nm, respectively.

2.3 Sol-gel / copolymer composite-wound dressings

2.3.1 Preparation of silica granules

Silica xerogel was prepared at room temperature via a one-step acid-catalyzed sol-gel process. TEOS, desired DI and HCl were mixed and stirred to form an acid-catalyzed sol. The pH of sol was 2.3. The methanol solution was added into the acid-catalyzed sol. Upon mixing, the sol was cast into cylindrical polystyrene vials. The vials were sealed and the sols were allowed to gel and age at 37°C. Subsequently, the vials were opened, and the gels were allowed to dry in an oven at 37°C until the gel weight became constant. The dried xerogel disks were then crushed into granules using mortar and pestle. The crushed silica granules were sieved by nylon meshes to obtain granules with the range 20-105 μ m.

2.3.2 Copolymers: materials and synthesis

Pyridine, 99% was purchased from Acros, polyethylene glycol 1000 and bis(trichloromethyl) carbonate were purchased from Fluka, methylene chloride, HPLC grade and methanol, HPLC grade were supplied from Fisher Scientific, tetrahydrofuran, high purity solvent, stabilized with 250 ppm BHT, was purchased from EMD, 2-propanol, Dulbecco's phosphate buffer saline, acetonitrile HPLC grade and water solution containing 0.1% (v/v) trifluoroacetic acid, for HPLC were supplied from Sigma Aldrich. All the reagents were used as received.

The synthesis of tyrosine-PEG-derived polycarbonates has been previously described and the copolymer structure is shown in Figure 1.

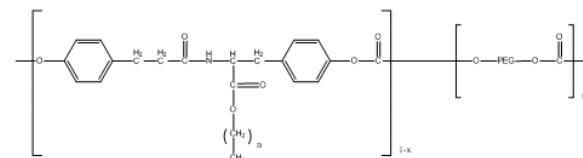


Figure 1. Structure of poly(DTR-co-x%PEG carbonate)s, where R is ethyl (n=1) or octyl (n=7)

2.3.3 Fabrication of composite film

Composites with 50% (w/w) xerogel were prepared by solution blending. Approximately 200 mg copolymer was dissolved in 0.75 ml tetrahydrofuran (THF) and 200 mg xerogel were added, followed by vigorous mixing for 1 min. The suspension was then poured into a small Petri dish, dried under nitrogen flow and in a vacuum oven at 40°C overnight. The resulting composite films were peeled off and compression molded into the desired

thickness. The xerogel loading was confirmed by TGA (results not shown here).

Composite films were prepared by compression molding, at temperatures 75°C above the respective T_g 's on a Carver press, followed by free cooling to room temperature. The two steel plates of the mold were covered with parchment paper in order to prevent the polymers and composites from adhering to the metal surfaces. For mechanical testing, shims with a thickness of 200 μm were used during the compression molding, while for the rest of the tests, the samples were pressed into 500 μm -thick films.

2.3.4 Water uptake, thermal and mechanical properties

Small disks of polymer and composite films (500 μm thick) were immersed in PBS at 37°C and at appropriate time points were removed from the media, wiped dry and weighed until they reached constant mass. The water uptake was calculated as $(m_f - m_i) * 100/m_i$ and was averaged for 3 replicates, where m_f and m_i are the mass of the sample after and before immersion, respectively.

The copolymer glass transition temperature (T_g) was determined by differential scanning calorimetry (2910 Modulated DSC, TA Instruments) as the midpoint of the transition in the second DSC scan. The decomposition temperature (T_d), defined here as the temperature at 2% mass loss, was measured by thermogravimetric analysis (2950 Hi-Res TGA, TA Instruments). Both DSC and TGA were performed on samples of 15 mg on average and at a heating rate of 10°C/min.

The thin (200 μm) polymer and composite films were tested according to ASTM standard D882-91 on a Sintech 5/D tensile tester. Measurements were done in dry state at room temperature, and also in water at 37°C after the samples were pre-incubated in PBS at the same temperature for 1, 3 and 5 days respectively. The results for Young's modulus, tensile strength at yield and at break, along with strain at break were averaged over four replicates. The initial grip speed was 2 mm/min allowing for a reliable measurement of the elastic modulus; the yield point was calculated based on the zero slope criterion.

3. RESULTS AND DISCUSSION

3.1 Sol-gel formulations for the controlled delivery of growth factor analog

Figures 2 and 3 show the release of dextran from acid-catalyzed (Figure 2) and acid-base catalyzed (Figure 3) sol-gels as a function of MW and immersion time in

PBS. The data demonstrate a long term, time-dependent release of dextran of various MW from both acid-catalyzed and acid-base catalyzed sol-gels.

The data also show that the rate of release varied with MW and processing parameters. The release rate from acid catalyzed sol-gels showed a noticeable reduction with increasing MW; by day 23, the amount released was 44, 28 and 16% for the molecules with a size equal to 10K, 40K and 70K, respectively. In addition, dextran of larger MW (40 and 70K) showed an early phase of delayed release. In contrast, the release of dextran from acid - base catalyzed sol-gels occurred at a higher rate and did not show any delayed release (Figure 3). In addition, the release profiles of dextran with various MW from the acid-base processed sol-gels showed a two-stage release. The initial faster release was followed by a slower, steady release of zero-order. The difference in the release from acid-catalyzed and acid-base-catalyzed sol-gels is most likely related to the difference in nanopore size. The initial delay in release of large molecules from the acid catalyzed sol-gels (pore size 1 nm) did not occur in the case of acid-base catalyzed gels which are mesoporous (pore size 2-5 nm). A larger pore size facilitates the release of larger molecules.

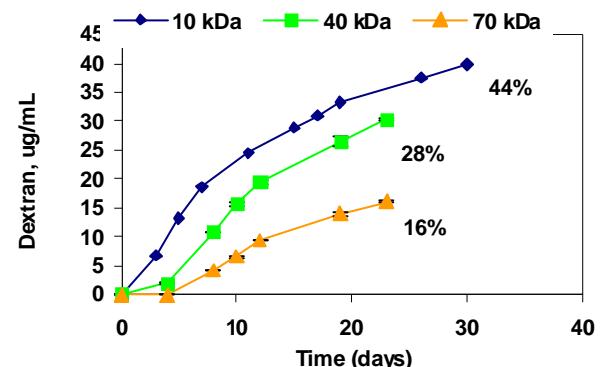


Figure 2. Release from acid-catalyzed sol-gels

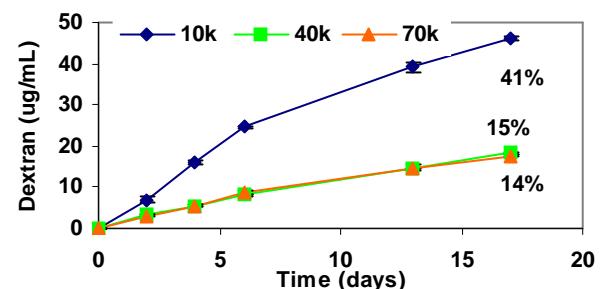


Figure 3. Release from acid-base catalyzed sol-gels

3.2. Sol-gel formulations for the controlled release of antibiotics

By using the sol-gel/emulsification procedure, drug-containing spheres with ideally smooth, defect-free surfaces were synthesized. The size of spheres varied with the speed of stirring during emulsification. In the speed range of 330-440 rpm, 60% of the spheres were in the range of 210-350 μm .

Cumulative release of vancomycin from microspheres (MS) is shown in Figures 4. The release from spheres is compared to the release from ground granules. In addition, Figure 4 shows the effect of R (R8 vs. R5) on the release of vancomycin. The data demonstrate a difference between the kinetics of release from microspheres and from ground granules. In contrast to a fast release of both drugs from ground granules, microspheres show a slower release. Whereas over 60% of the drug load was released from the granules within 3 days, the release of both drugs from spheres was extended over much longer period of time. The data also suggest that the release kinetics from the spheres are affected by the R.

The distinct release patterns between granules and microspheres could be associated with the differences in geometry and surface morphology. Possible differences in ultrastructure could also affect the release kinetics.

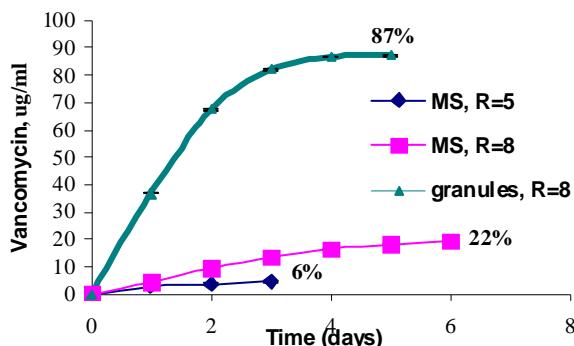


Figure 4. Cumulative vacomycin from granules and microspheres with different R

3.3 Sol-gel / copolymer composite-wound dressings

3.3.1 Water uptake and mechanical properties

Incremental addition of PEG to the polycarbonate structure changes the properties of the material quite significantly. On one hand, it increases the flexibility of the backbone, thus leading to a reduction in T_g and on the other hand it increases hydrophilicity of the copolymer, as can be seen from the gradual increase in the water uptake (Table 1).

Table 1. Glass transition temperature (dry state), decomposition temperature and water uptake of copolymers and composites

Copolymers and Composites	T_g (°C)	T_d (°C)	Water uptake (%)
DTO-5%PEG	25	334	4
DTO-10%PEG	2	342	17
DTO-20%PEG	-19	343	65
DTO-40%PEG	-38	341	210
DTE-20%PEG	4	338	73
DTO-20%PEG-50%R10-3.5%BP	-18	N/A	27
DTO-10%PEG-50%R10-3.5%BP	4	N/A	11

While some of the copolymers used in our study can triple their mass upon immersion in PBS, all of them absorb significantly less water than the commercially available FlexiGel®, which absorbs up to 526%. The relationship between the PEG mol fraction in the copolymers and water uptake is non-linear, as previously observed for DTE copolymers (Yu et al., 1999). However, the water uptake for the DTE-based copolymers is much steeper compared to DTO-based polycarbonates because the octyl group renders the copolymers more hydrophobic and also because, for the same mole percent of PEG in copolymer composition, the mass fraction of PEG is larger for DTE copolymers compared to DTO copolymers due to their respective monomer molecular weights.

Addition of soft, flexible PEG units in the polycarbonate backbone significantly reduces the tensile modulus from 304 MPa for 5% PEG1K to 0.98 MPa for 20% PEG1K. When R_{10-100} (20-105 μm) xerogel is added to the copolymer, the composite films become much stronger than their respective copolymers, with the Young's modulus increasing by an order of magnitude (Figure 5) but they remain soft and flexible for the formulations containing 10% and 20% PEG1K. The strain at break for the composite undergoes a significant reduction, especially for the 20% PEG containing composite.

However, since wound dressings are exposed to body fluids, it is important that the polymeric films maintain their physical integrity in the wet state for the period of time they are kept on the wound (in our case for up to 3 days). Upon incubation of the copolymers in PBS, the absorbed water acts as a plasticizer and decreases dramatically their moduli by up to two orders of magnitude after the first day. From day 1 to 3 and 5, the drop-off in modulus for composites continues gradually, but to a much smaller extent (Figure 6). While the polymer matrix is able to swell considerably (65% and 210%, for 20 and 40% PEG-containing copolymers, respectively), the xerogel particles do not, possibly

leading to de-bonding and explaining the big initial drop in strength for these composites in the wet state. Additionally, the xerogel particles degrade hydrolytically over time (Falaize et al., 1999), which may explain the weakening observed from day 1 to day 5 of incubation. This de-bonding could also be responsible for the similar trend observed for the strain at break and we can conclude that from the mechanical point of view the composite films meet the requirements for use as wound dressings.

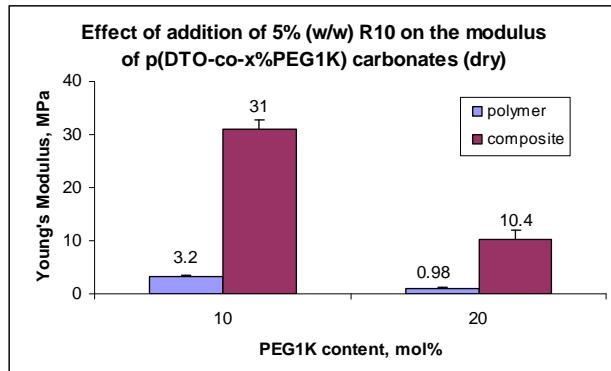


Figure 5. Young's modulus of dry samples of copolymers and their respective composites with 50% (w/w) R_s10-100 (20-105 μ m) xerogel

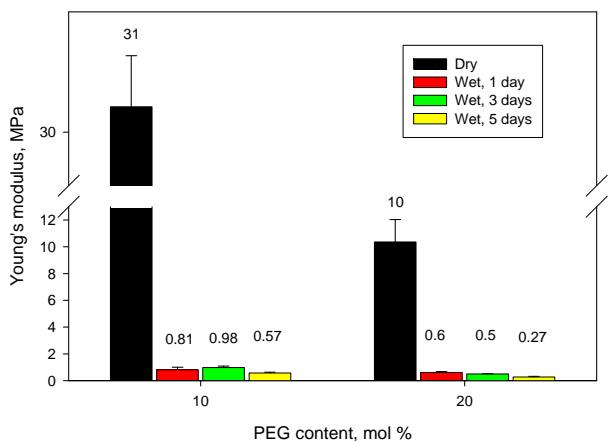


Figure 6. Young's modulus of composite films in wet and dry state

CONCLUSIONS AND FUTURE WORK

This study has shown that the release kinetics of dextran simulating large molecules and antibiotics from sol-gel particles can be tailored by variation of the synthesis parameters. Composites from p(DTR-co-PEG carbonate)s and sol-gel particles were successfully prepared by solution blending with inorganic loadings as high as 75% w/w. Addition of these reinforcing particles has beneficial effects on the mechanical properties of the composite, especially on the hydrated samples. As a result, both initial modulus and strain at break compare favorably with commercially available hydrogel wound dressings.

Based on these initial studies, our future study will include the incorporation of growth factors to sol-gel particles and its controlled release from wound dressing composites. Taken together, we hypothesize that these studies will advance a promising approach to the complex therapeutic issues surrounding CS in today's wounded warrior.

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